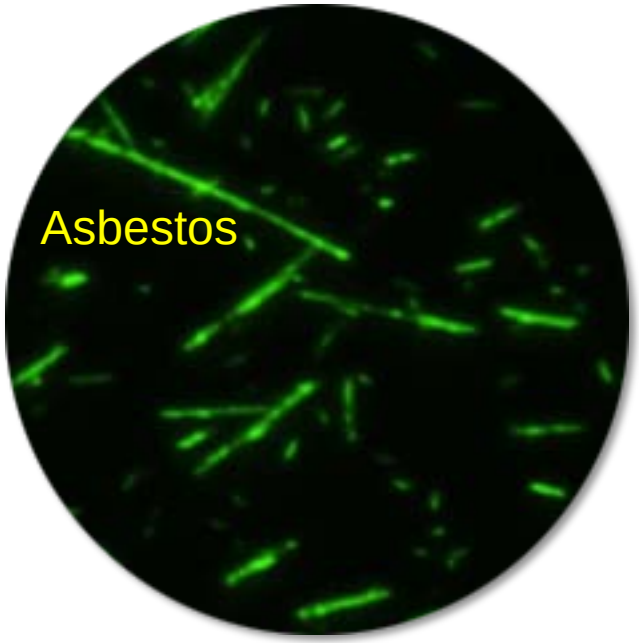
NEMC2011(Seattle) Aug. 18, 2011

Detection of Airborne Asbestos by Fluorescent-labeled protein probe and its Application to quick Monitoring



Fluorescent microscopy

*Akio Kuroda,
Takenori Ishida,
Maxym Alexandrov,
Tomoki Nishimura

Hiroshima University, Japan

Asbestos: silicate mineral fiber

serpentine

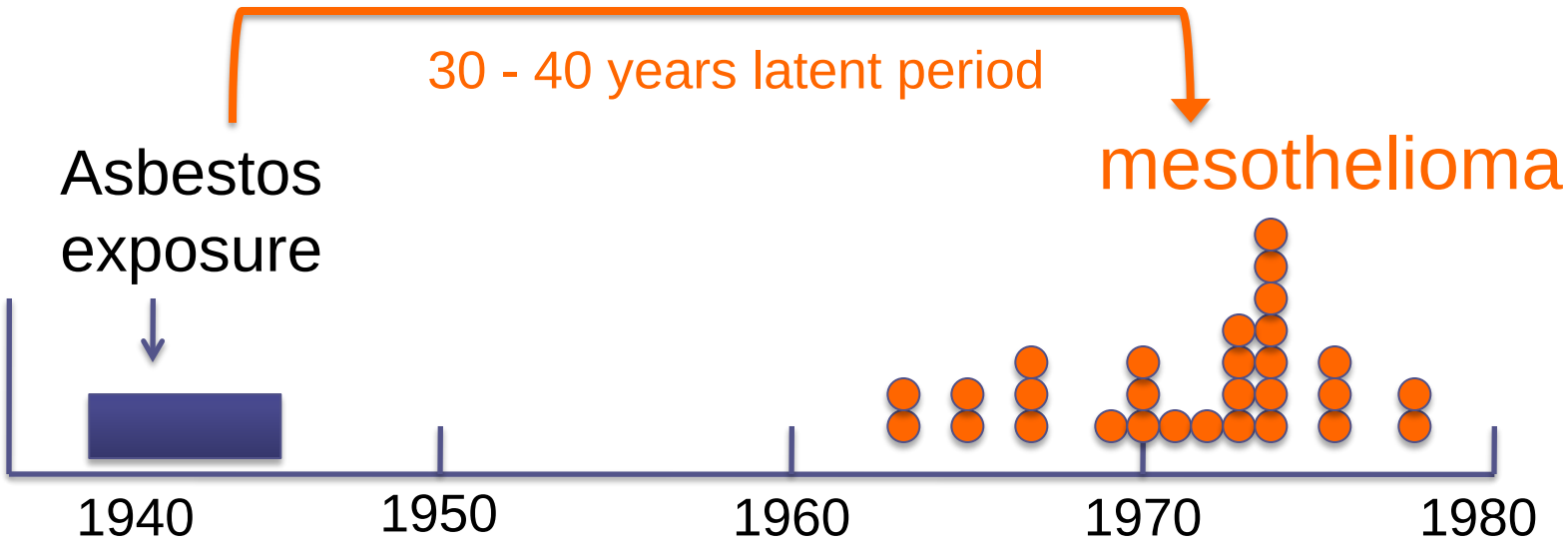
amphibole

Asbestos: widely used in construction materials

東日本大震災NEVER特設ページより

Asbestos: lung cancer and mesothelioma

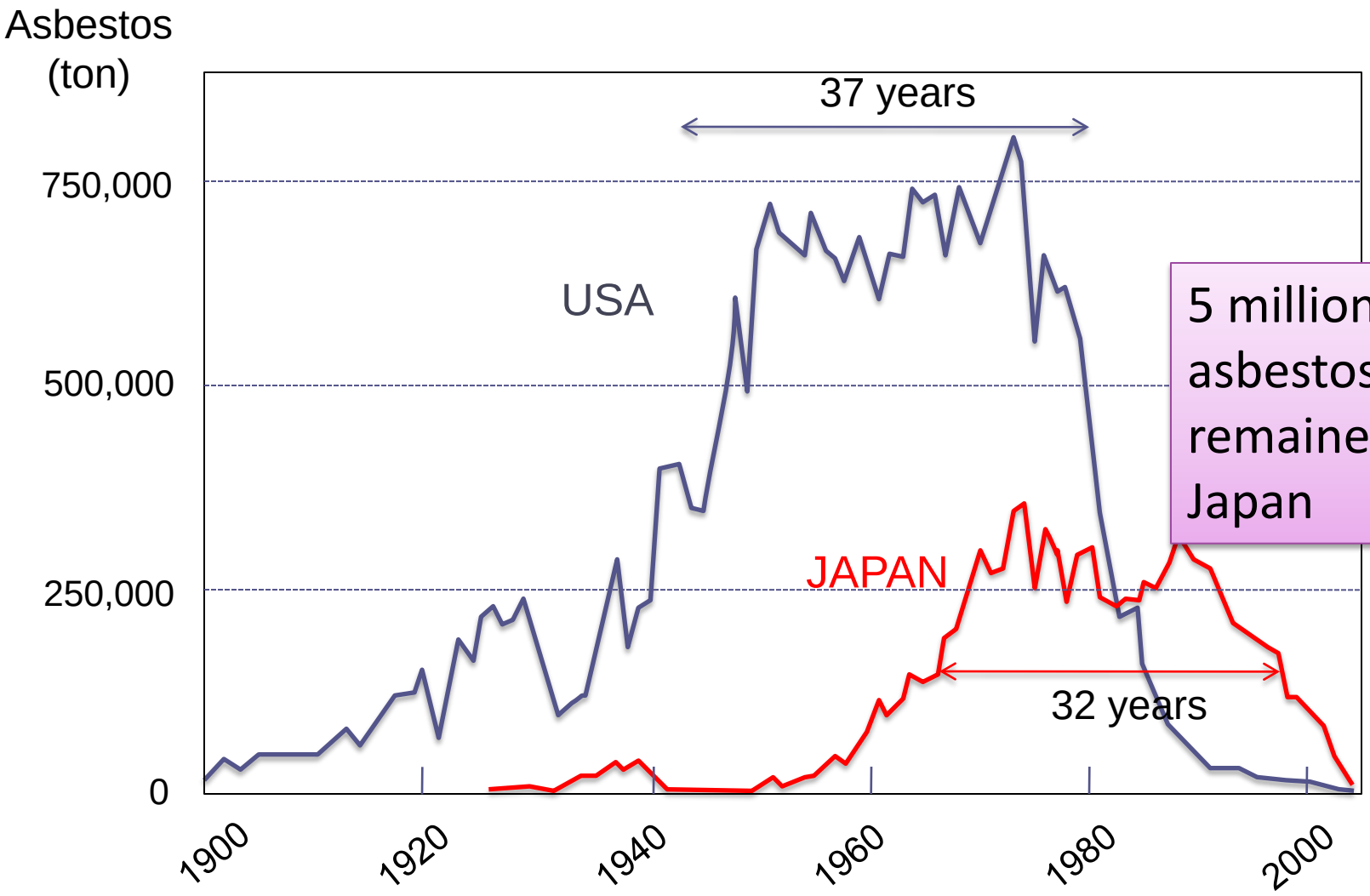
Johnes, IARC Sci Publ. 1980;(30):637-53.1980



Japan shock 2005

This has come largely as a shock to the Japanese public in 2005.
Not all those deaths were workers; many of the deaths were people who lived near factories including family members of workers.

Amount of asbesots in USA and Japan



5 million tons of asbestos are remained in Japan

Worldwide trends in the mesothelioma

The NEW ENGLAND JOURNAL of MEDICINE

Table 1. Worldwide Trends in the Epidemiologic Features of Malignant Mesothelioma.*

Country or Region	Incidence	Predicted Peak Year	Predicted No. of Deaths in Next 40 Yr†	Predicted Cost‡
	cases/million population			*billions of U.S. dollars*
United States	15	2004	72,000	200
Europe	18§	2015–2020	250,000	80
Japan	7	2025	103,000	—
Australia	40	2015	30,000	5–10

* The sources of the data on the incidence (most recent figures), predicted peak year, and predicted number of deaths in the next 40 years are as follows: United States, Roushdy-Hammady et al.[2]; Europe, Pelin et al.[3]; Japan, Sebastien et al.[4]; and Australia, Wagner et al.[5] The sources of the data on predicted cost are as follows: United States, Shah and Williams[6]; Europe, Lee et al.[7]; and Australia, Wagner et al.[5] Costs for Japan are unknown.

† The predicted number of deaths is estimated from data on annual incidence and predicted peak year.

‡ The costs shown are for compensation only; health care costs are excluded.

§ The incidence, in number of cases per million population, is 33 in Great Britain, 30 in the Netherlands, 15 in Germany, 16 in France, and 19 in Italy (range in Europe, 15 to 33).

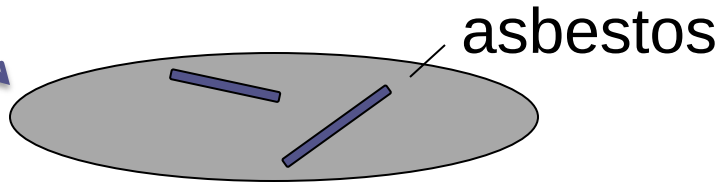
Robinson and Lake, N Engl J Med 2005;353:1591-1603.

Why rapid detection method of asbestos is required?

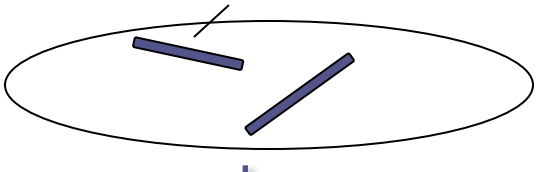
Conventional method for airborne asbestos

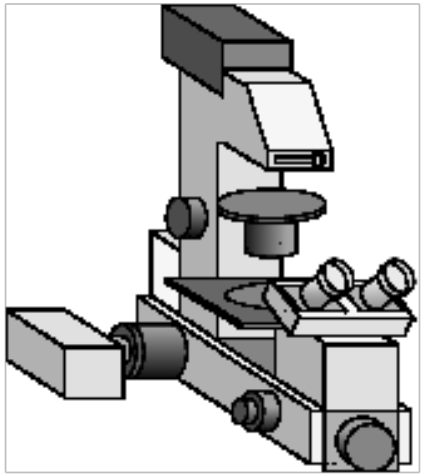
Membrane filter

Pump



Treat with acetone vapor (transparency)





Phase contrast microscopy (PCM)

Phase contrast microscopy (PCM)

Asbestos monitoring (Ministry of Environment)

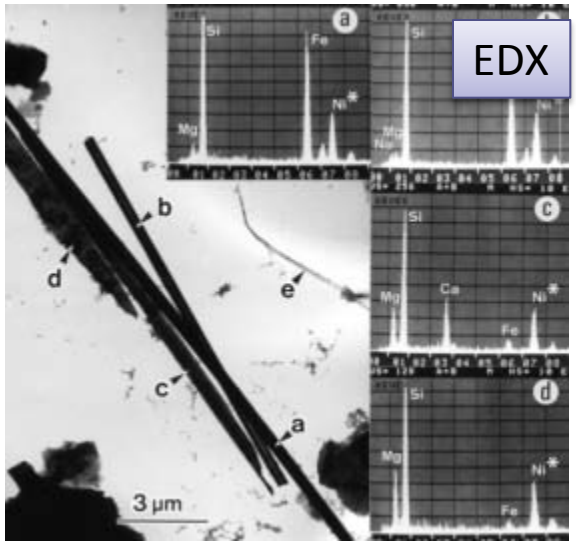
【Phase contrast microscopy】

Total fiber concentration under PCM

Less than 1 fiber /L → OK

More than 1 fiber /L → Asbestos or not ? under electron microscopy

【Electron microscopy wit EDX】



Time consuming and expensive

Asbestos risk at demolition site

Past

Asbestos factory

At present

Demolition site

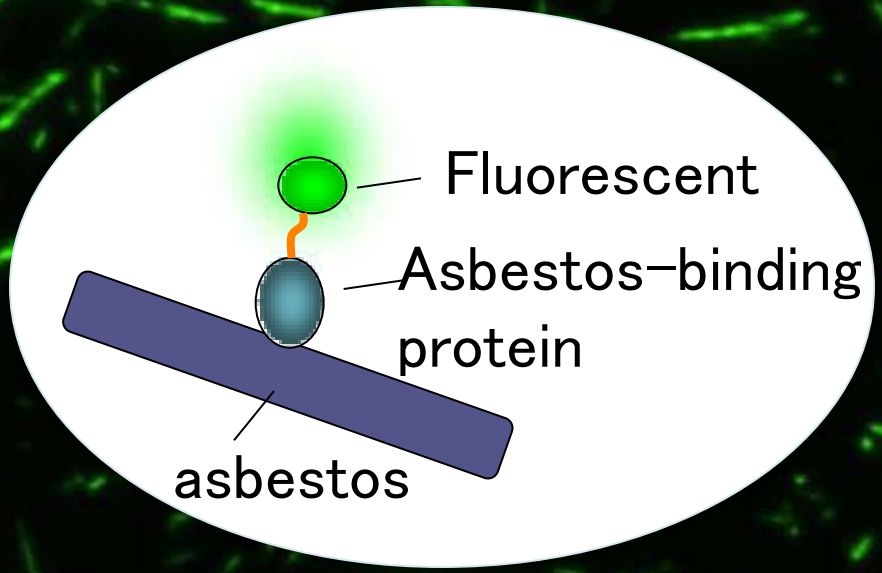
Asbestos risk at demolition site

- ✓ More than 100 million tons of materials containing asbestos will be dumped until 2035.
- ✓ Demolition will be completed within a couple of days
- ✓ Rapid detection method of asbestos is required

Airborne asbestos from earthquake debris？

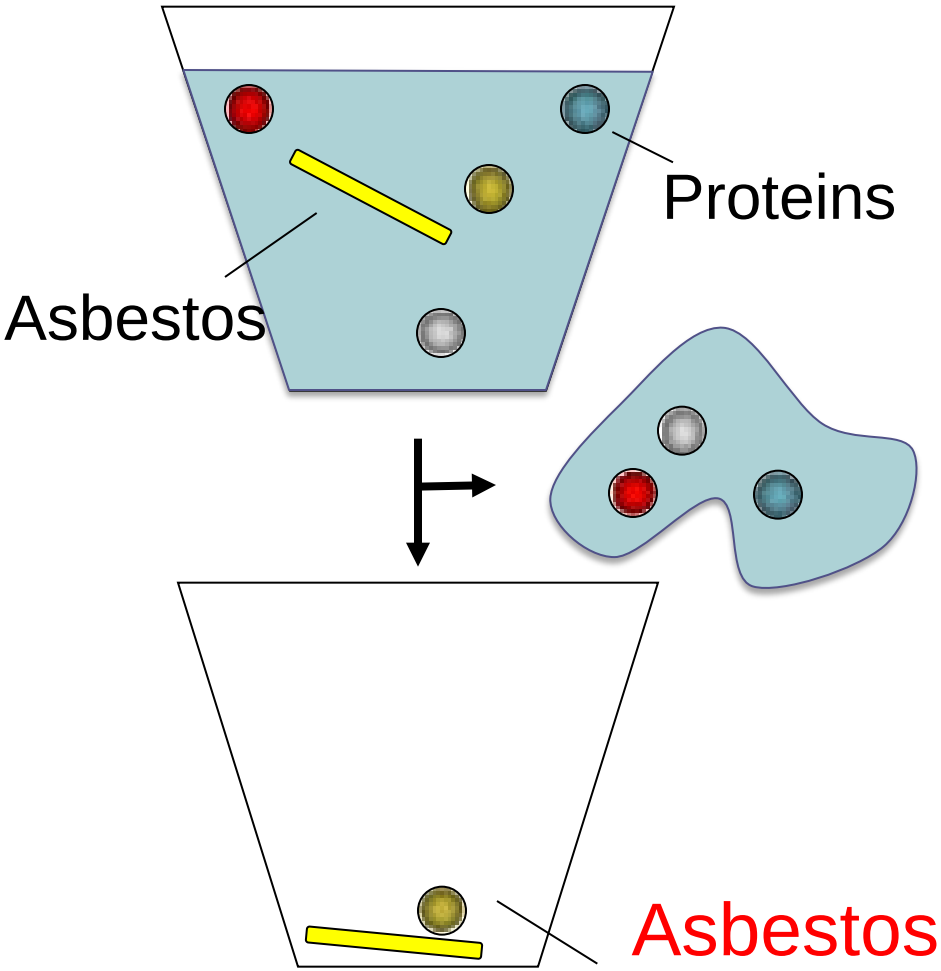
Tagajyo city, Miyagi Pref.

Quick detection of asbestos



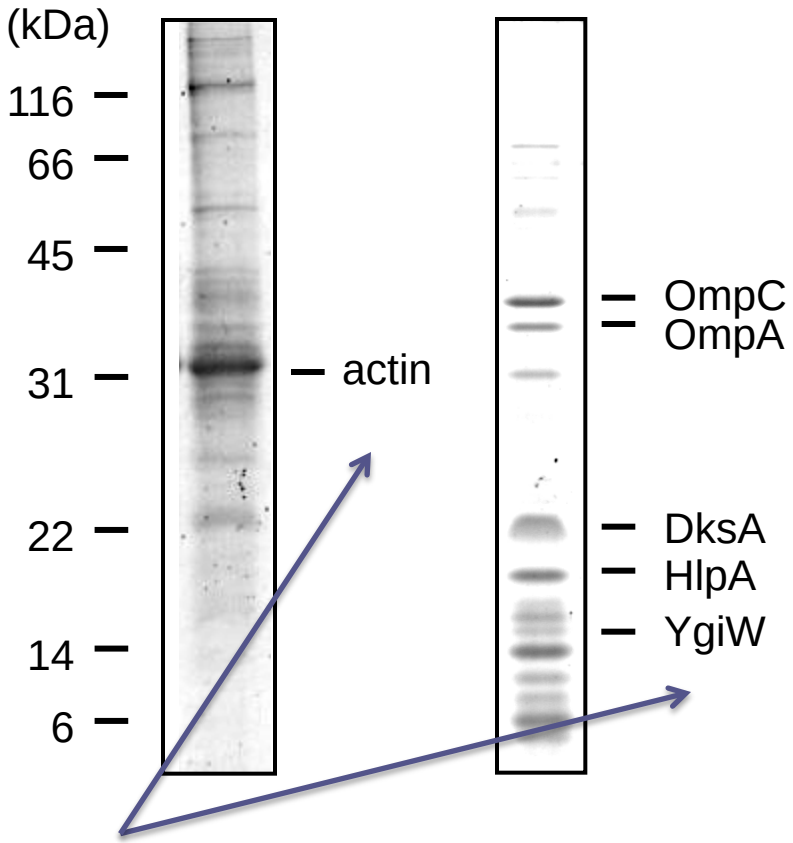
Asbestos-binding proteins

Asbestos-binding protein



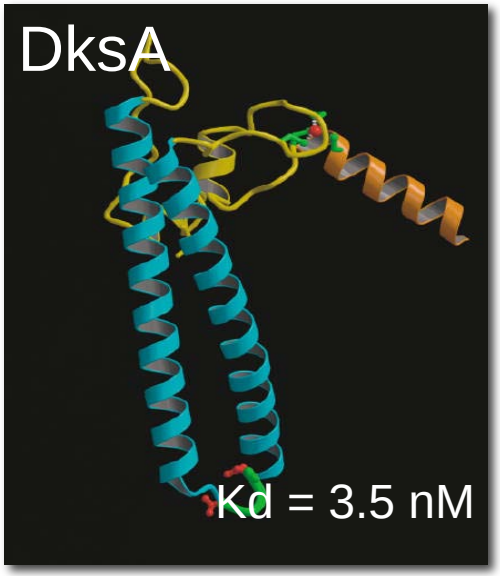
Protein sources

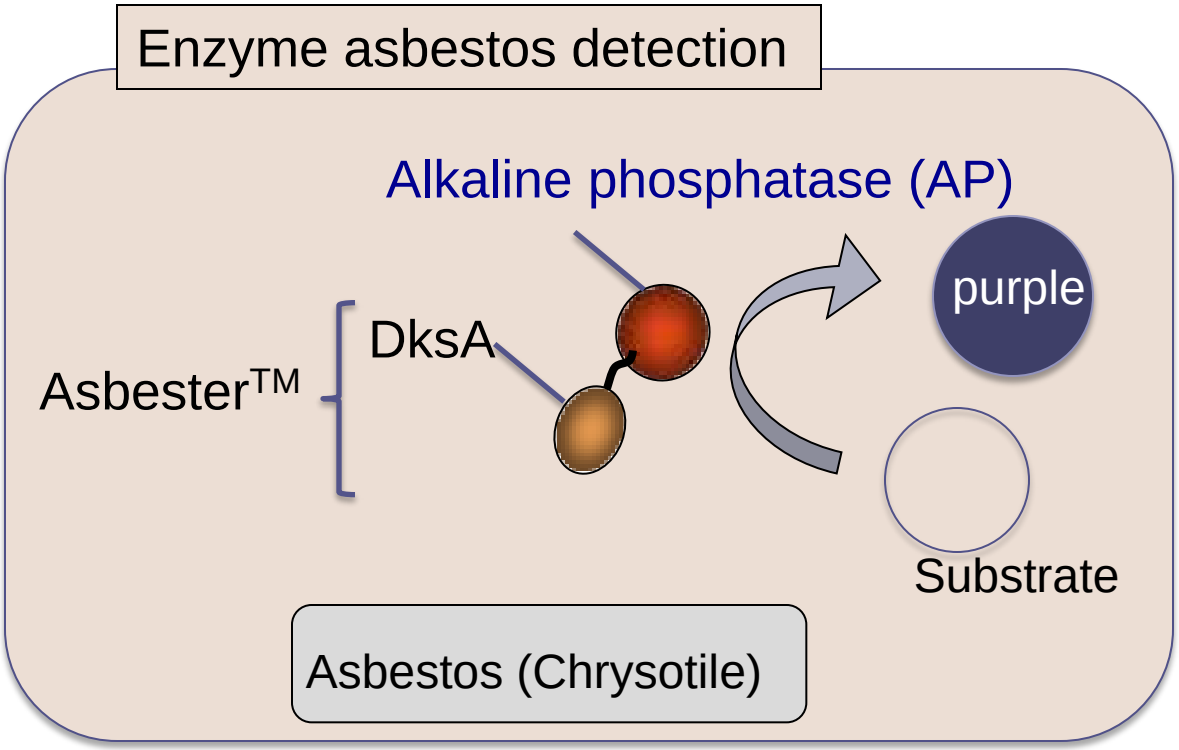
Mouse lung　Escherichia coli



Asbestos-binding protein！

DksA: chrysotile-binding protein



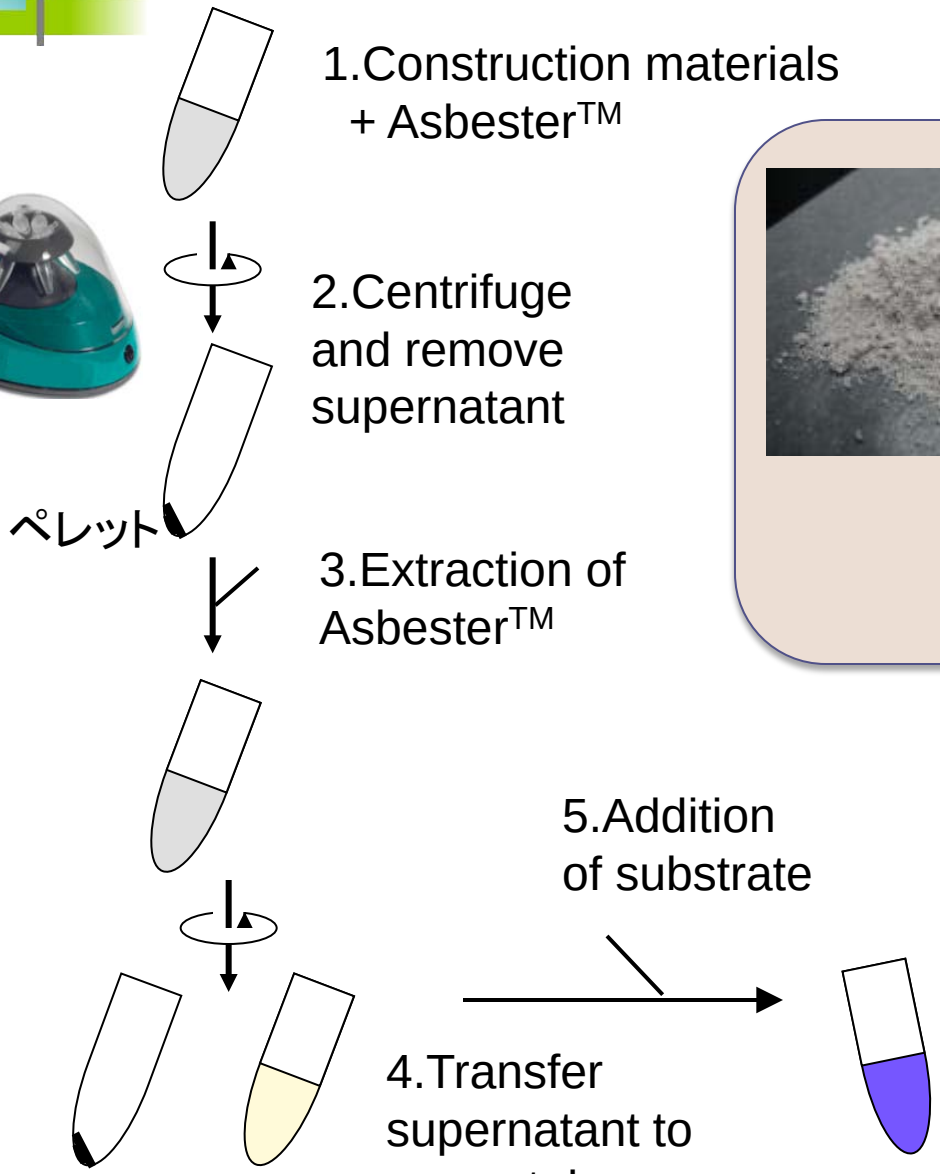


Asbestos (Chrysotile)

0 0.005 0.01 0.05 0.1 0.5 1 (mg)

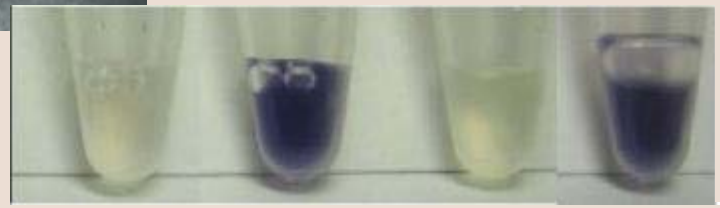
Chrysotile detection in the materials

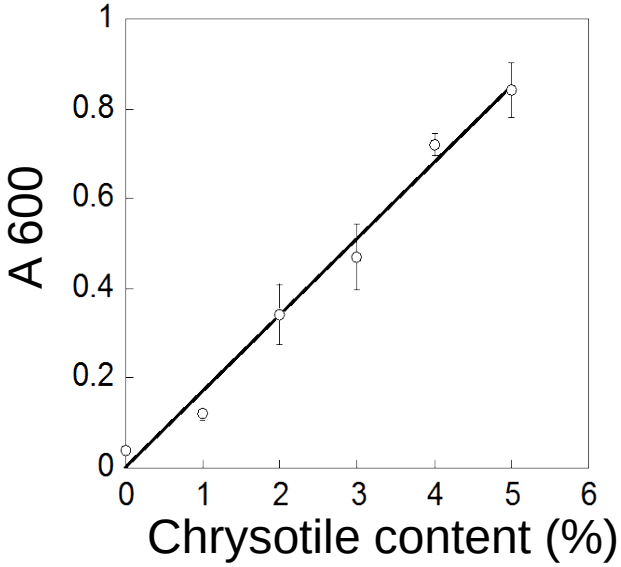
広島大学



Example

Dry wall (asbestos or non-asbestos)





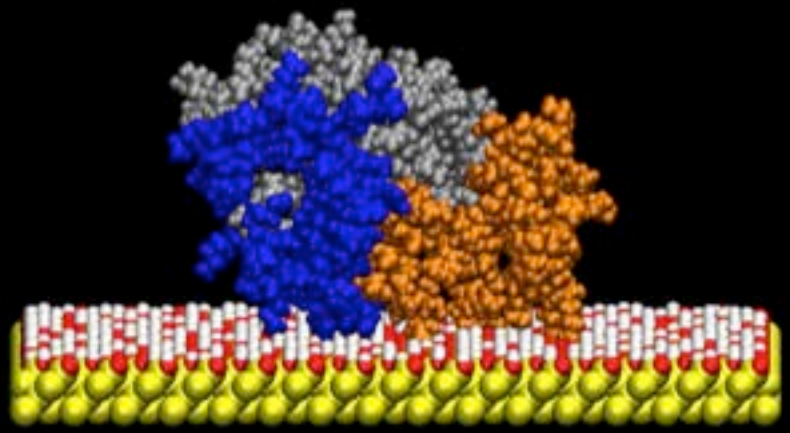
Specificity of Asbester (DksA-AP fusion)

Indistinguishable by X-ray diffraction

Materials	Compos	Bind
Chrysotile	$Mg_6Si_4O_{10}(OH)_8$	+
Antigorite	$Mg_6Si_4O_{10}(OH)_8$	-
Talc	$Mg_3Si_4O_{10}(OH)_2$	-
Amosite	$(Mg,Fe)_7Si_8O_{22}(OH)_2$	-
Crosidolite	$NaFe^{+3}{}_2Fe^{+2}{}_3Si_8O_{22}(OH)_2$	-
Glass wool	$CaO\text{-}P_2O_5\text{-}SiO_2\text{-}Al_2O_3$	-
Silica	SiO_2	-
Titanium oxide	TiO_2	-
Silicon carbide	SiC	-
Magnesium hydro	$Mg(OH)_2$	-(-/+)
Cement	SiO_2, CaO, etc	-
Rockwool	SiO_2, CaO, etc	-
Dry wall	$CaSO_4$	-

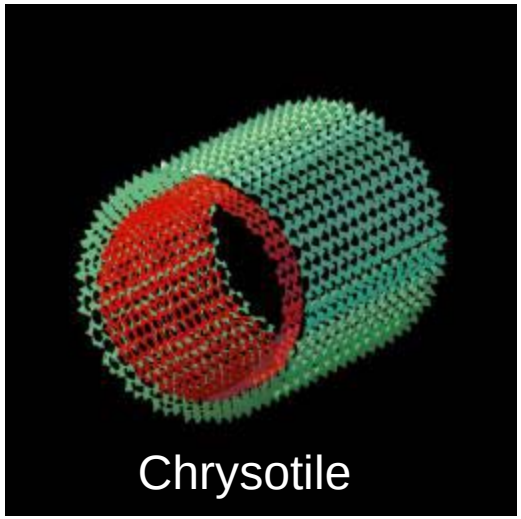
(b) after 250 ps

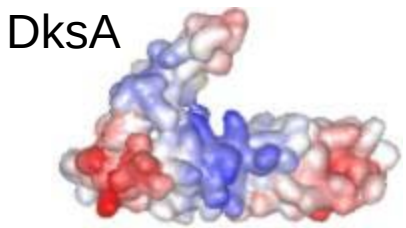
(d) after 3 ns

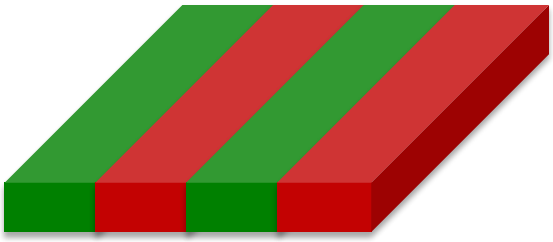


Tosaka, et al., Langmuir 2010, 26(12), 9950–9955

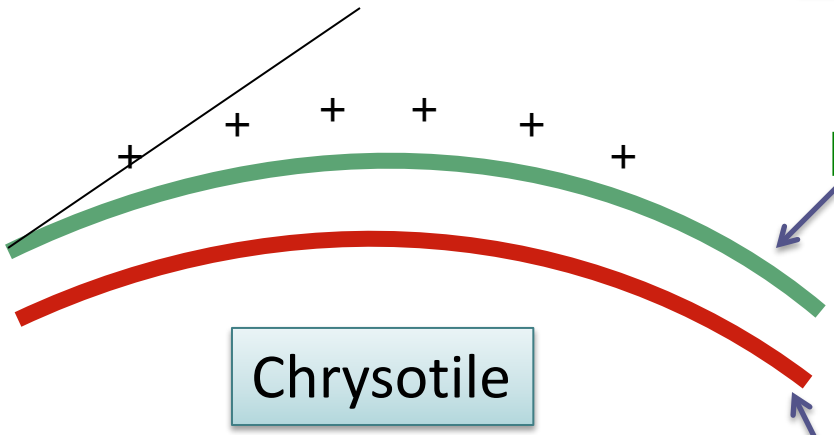
How does DksA recognize asbestos?







5.5 nm

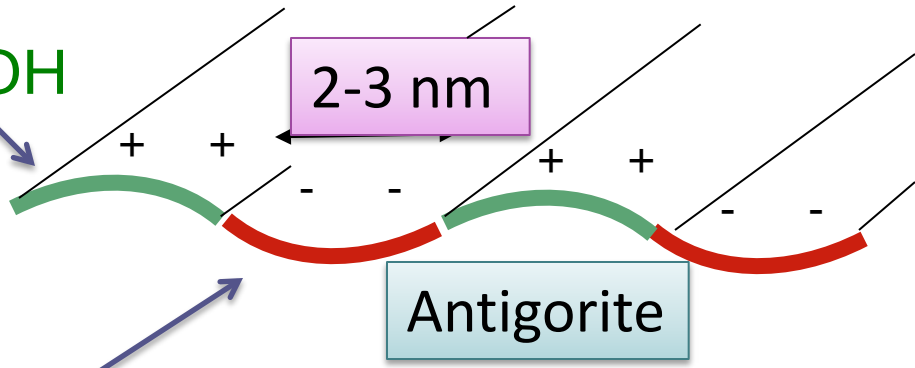


$Mg_6Si_4O_{10}(OH)_8$

Mg-OH

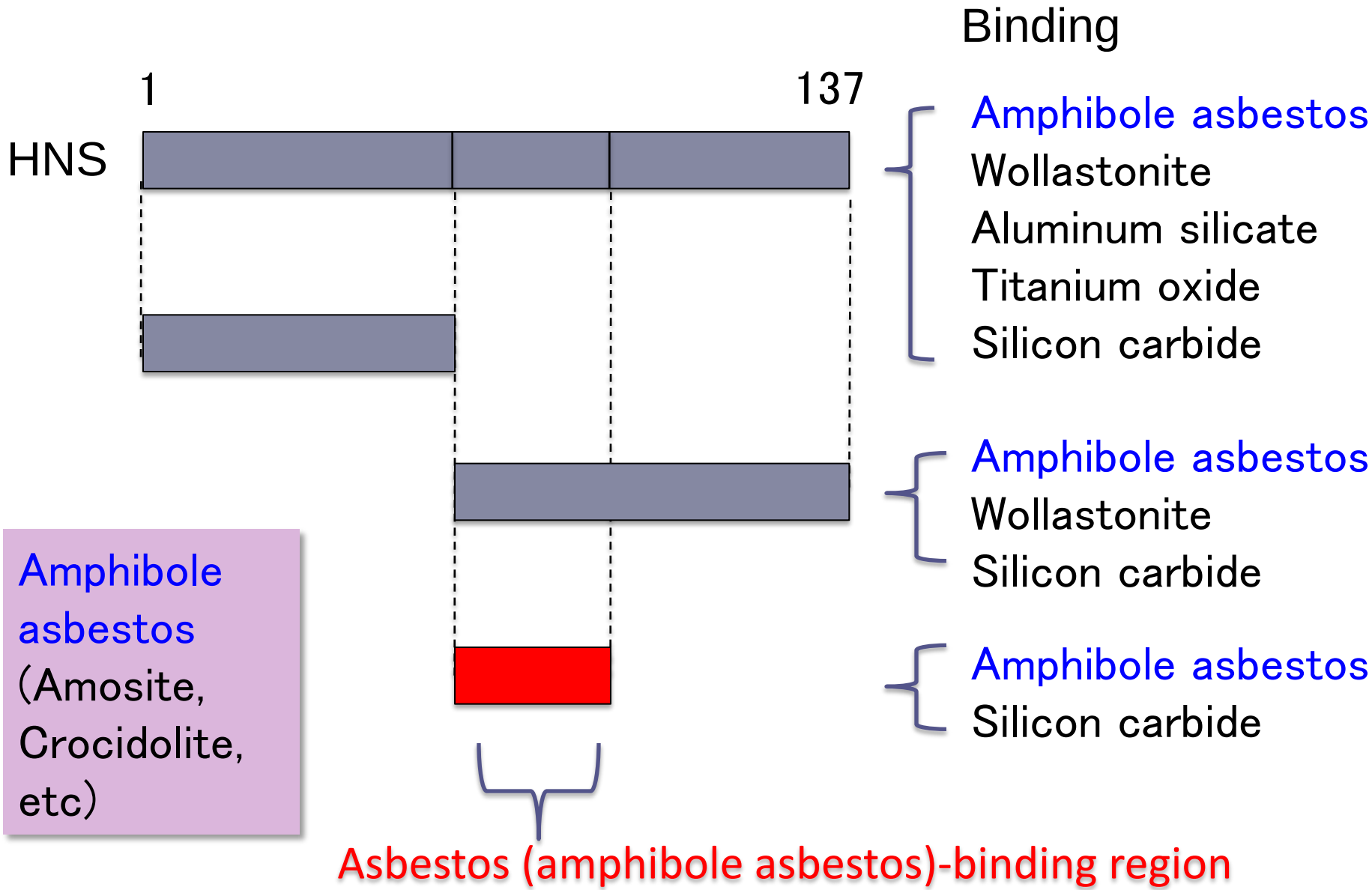
SiO_2

2-3 nm



$Mg_6Si_4O_{10}(OH)_8$

HNS: amphibole asbestos-binding protein

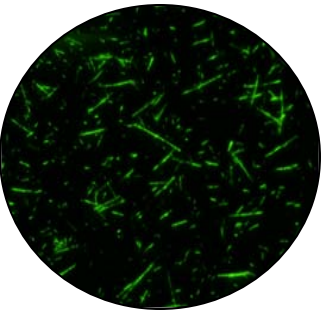


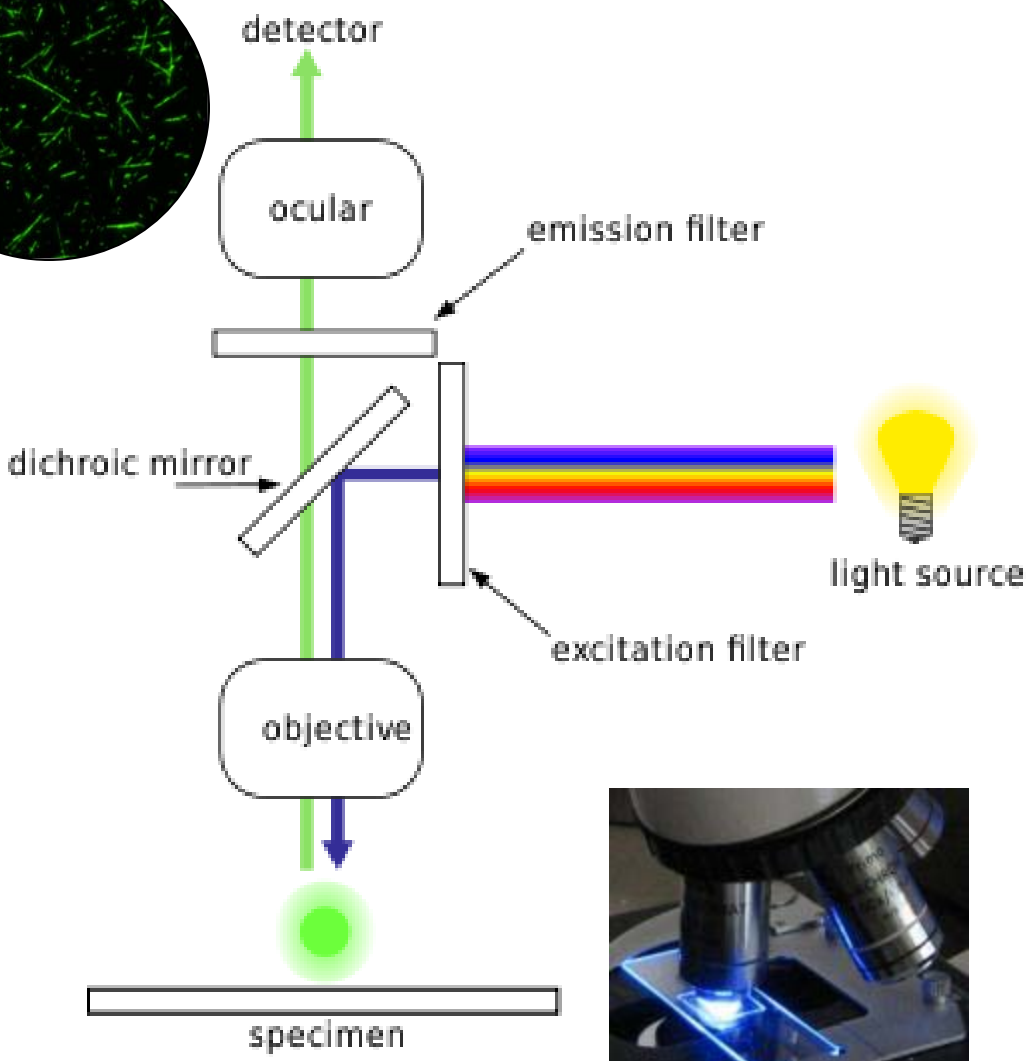
Specificity

	Fiber	DksA	HNS (modified)
Asbestos	Chrysotile	Bound	
	Crocidolite		Bound
	Amosite		Bound
	Anthophyllite		Bound
	Tremolite		Bound
	Actinolite		Bound
Non-asbestos	Glass wool		
	Fine glass fiber		
	Rockwool		
	Fire proof fiber (RF1)		
	Fire proof fiber (RF2)		
	Aluminum silicate fiber		
	Titanium potassium		Slightly bound
	Silicon carbide whisker	Bound	Bound
	Titanium oxide whisker		
	Wollastonite		

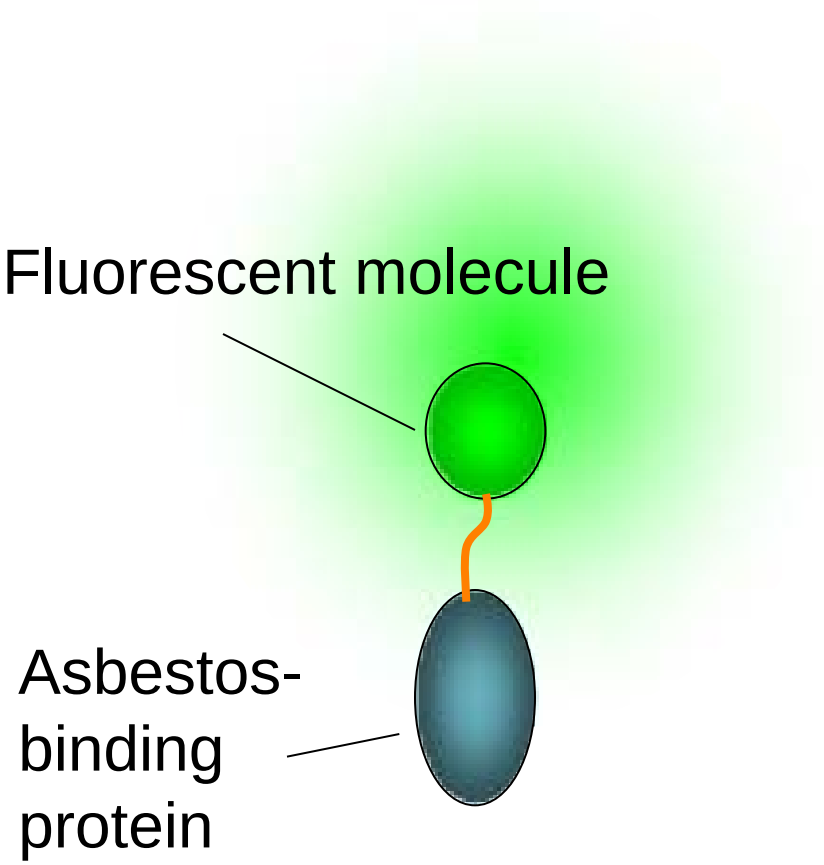
Detection of asbestos under fluorescence microscopy

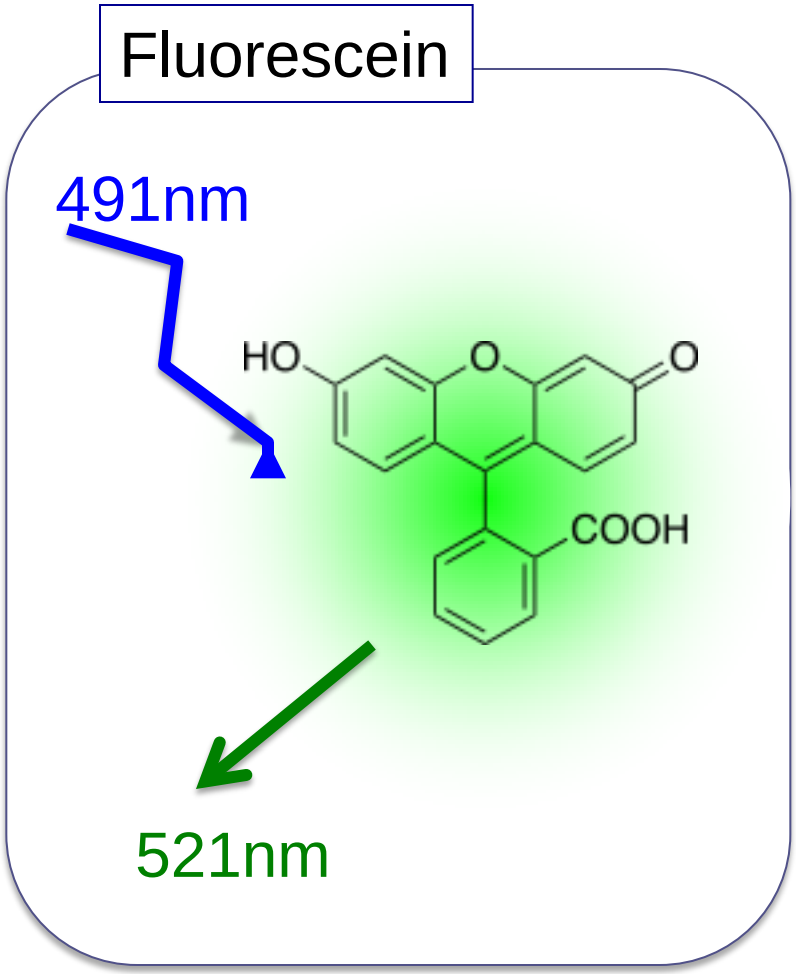
Fluorescent microscopy (FM)





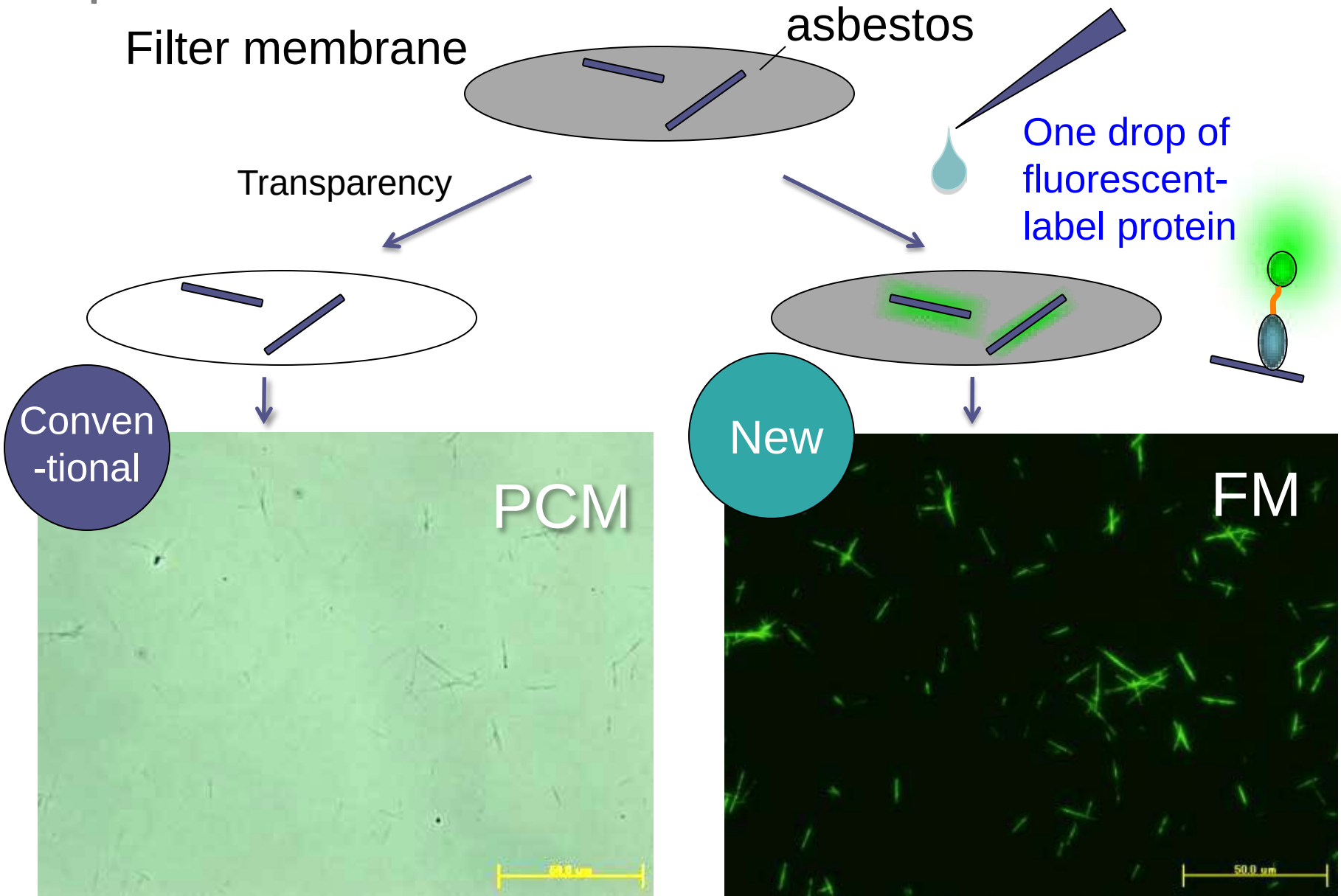
Modification of protein with fluorescence

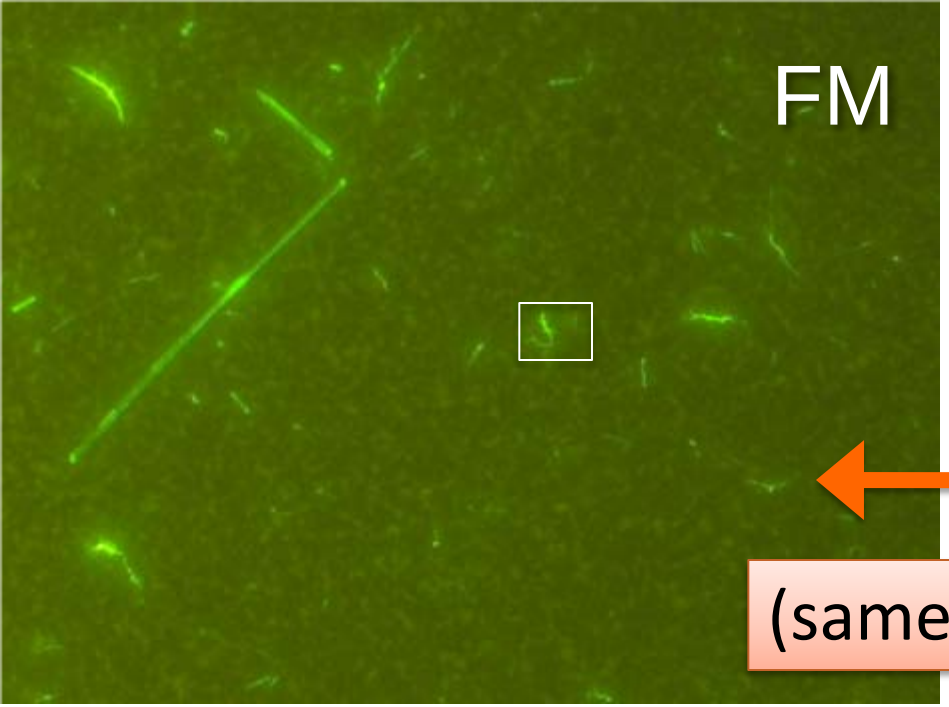


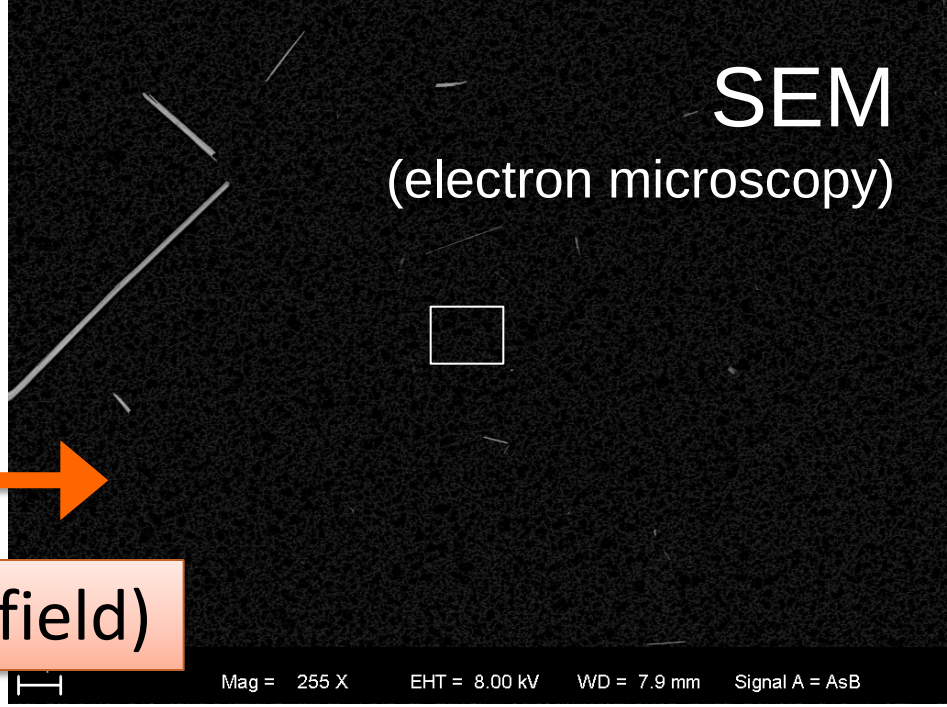


Asbestos

Detection of airborne asbestos under FM

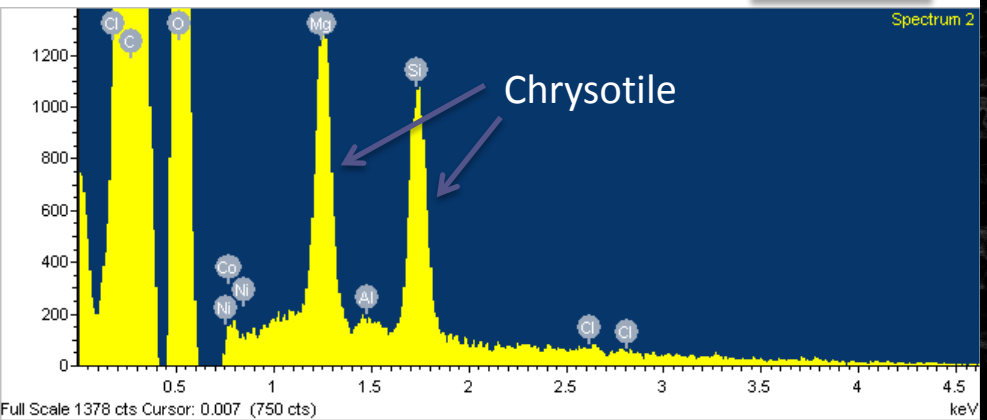


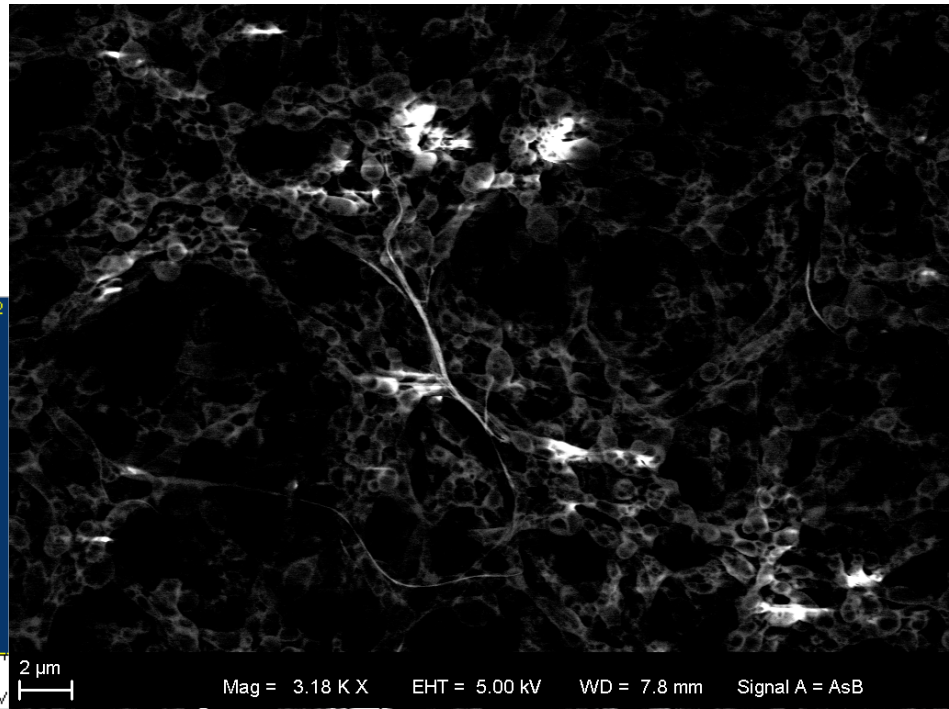


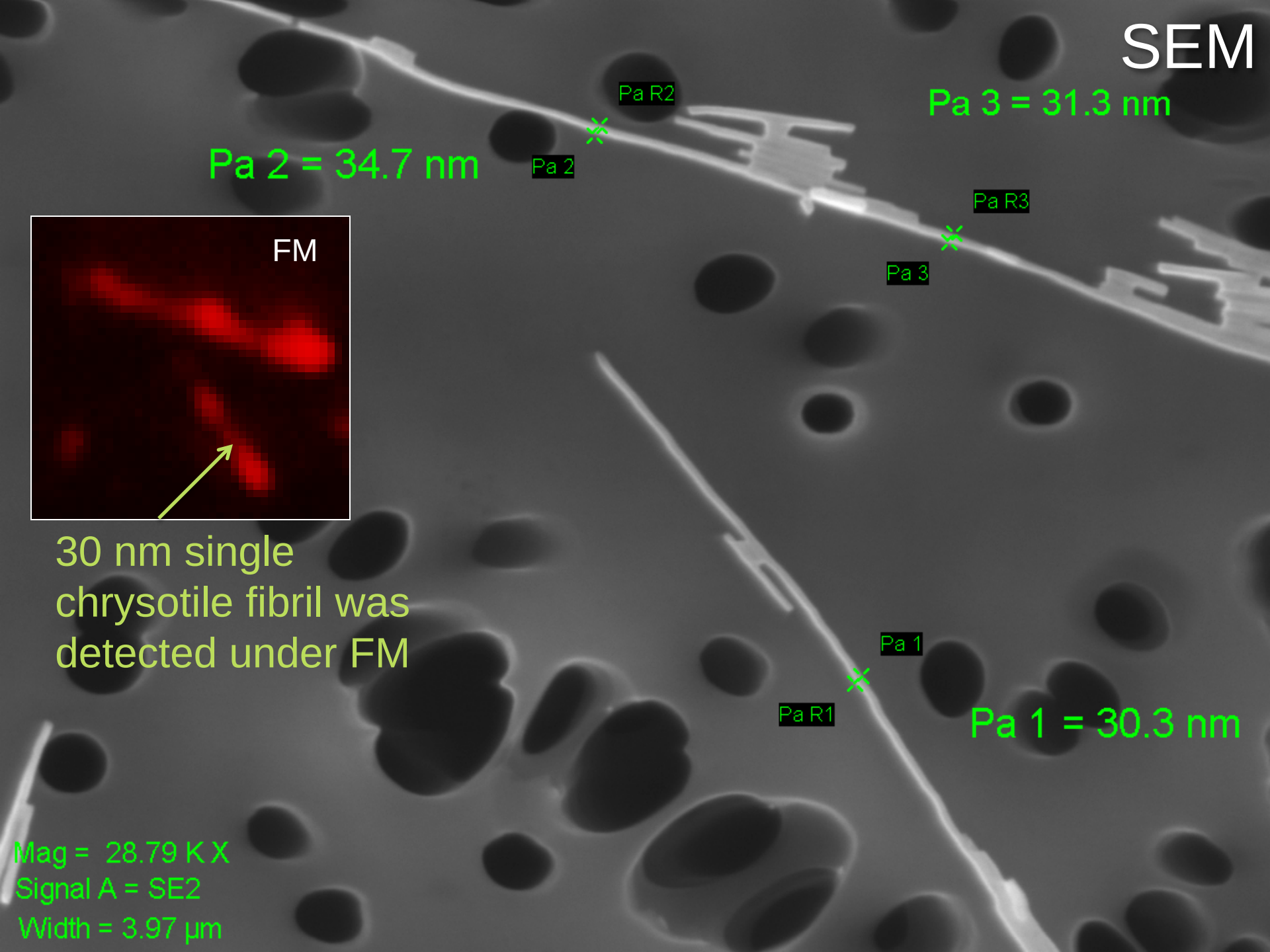


(same field)

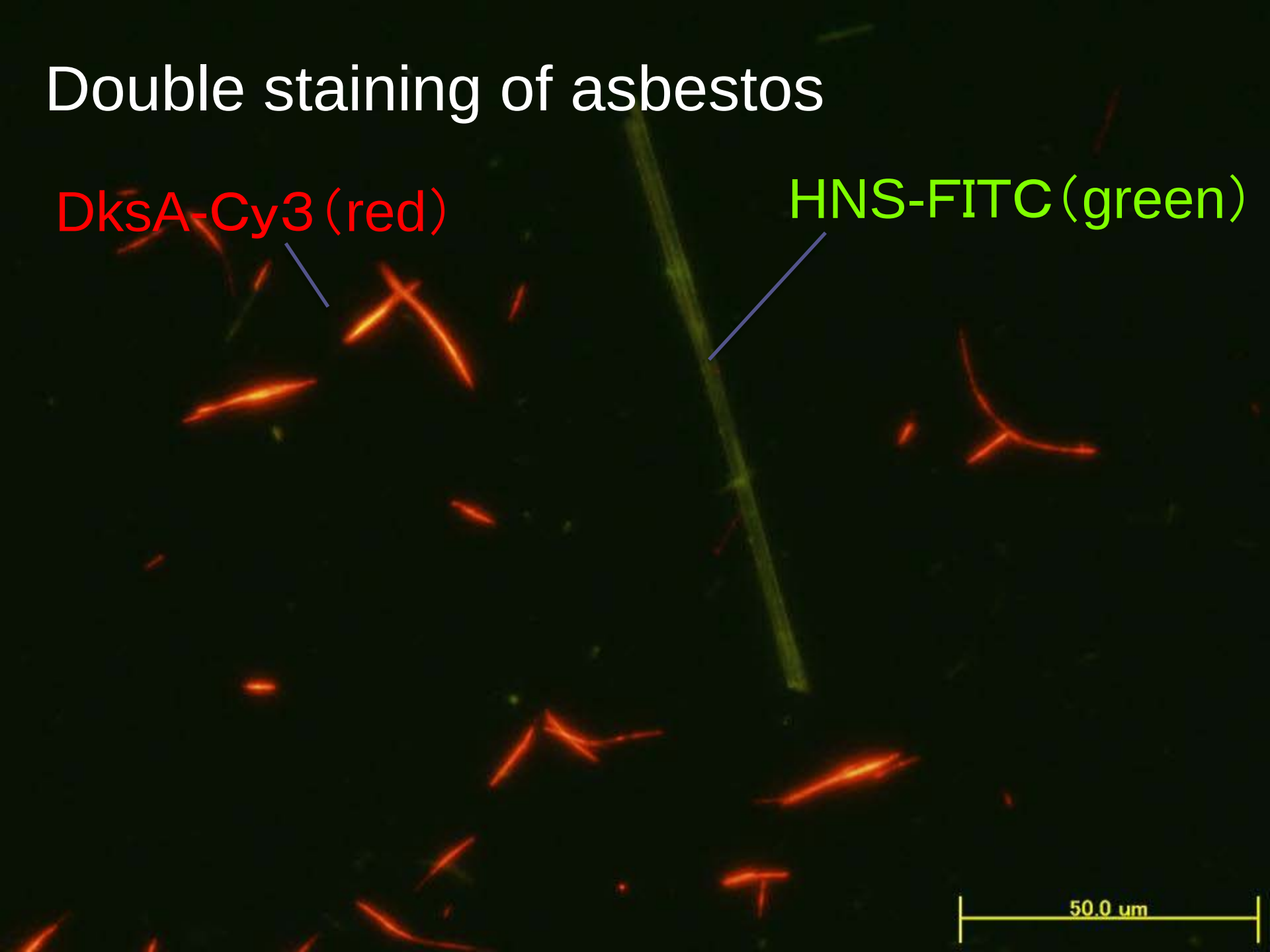
EDX






SEM
Pa R2
Pa 3 = 31.3 nm
Pa 2 = 34.7 nm
Pa 2
Pa R3
Pa 3
FM
30 nm single chrysotile fibril was detected under FM
Pa 1
Pa R1
Pa 1 = 30.3 nm
Mag = 28.79 K X
Signal A = SE2
Width = 3.97 µm

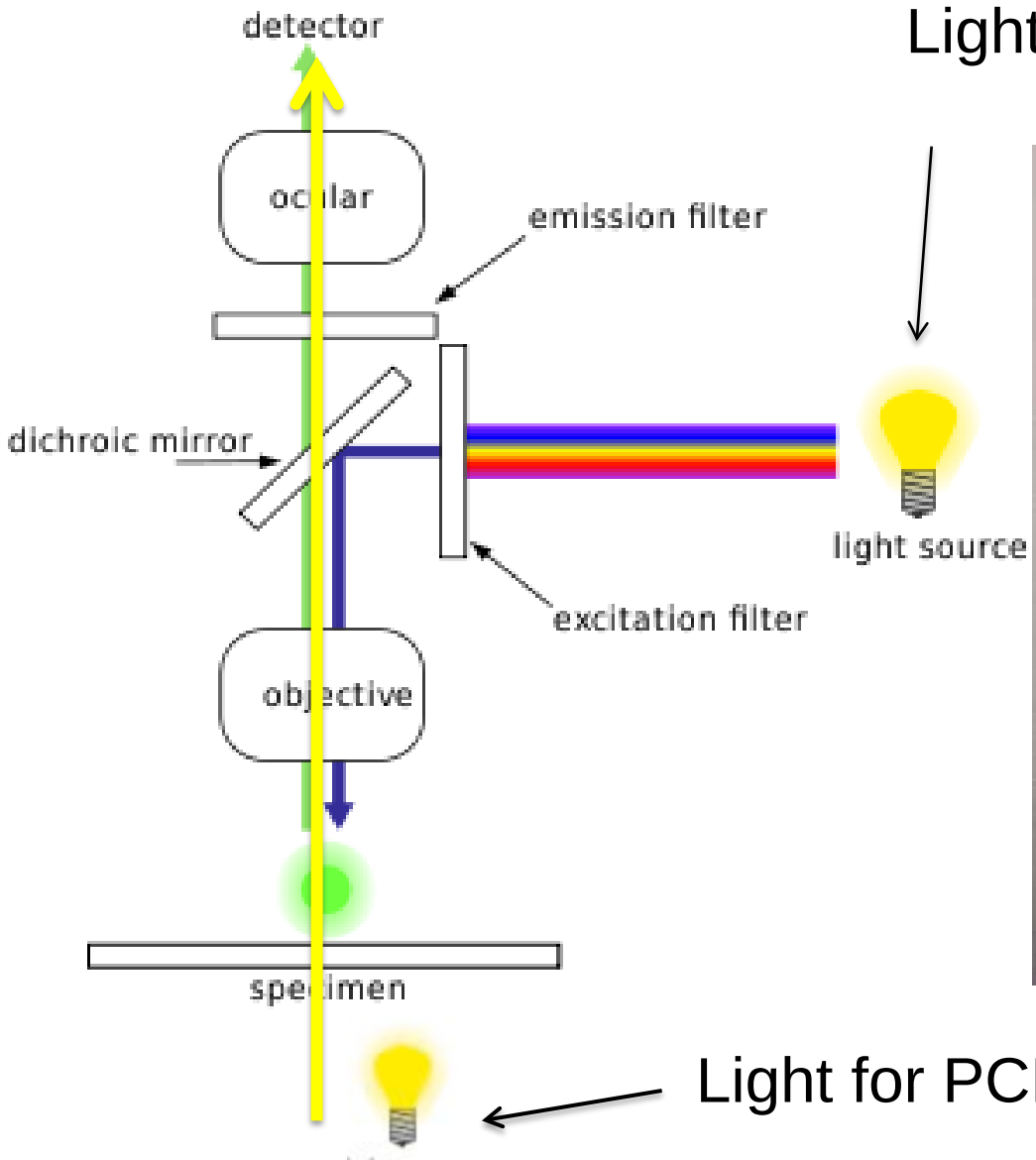
Double staining of asbestos



Combination of phase-contrast and fluorescence microscopy (PCM-FM fusion)


PCM

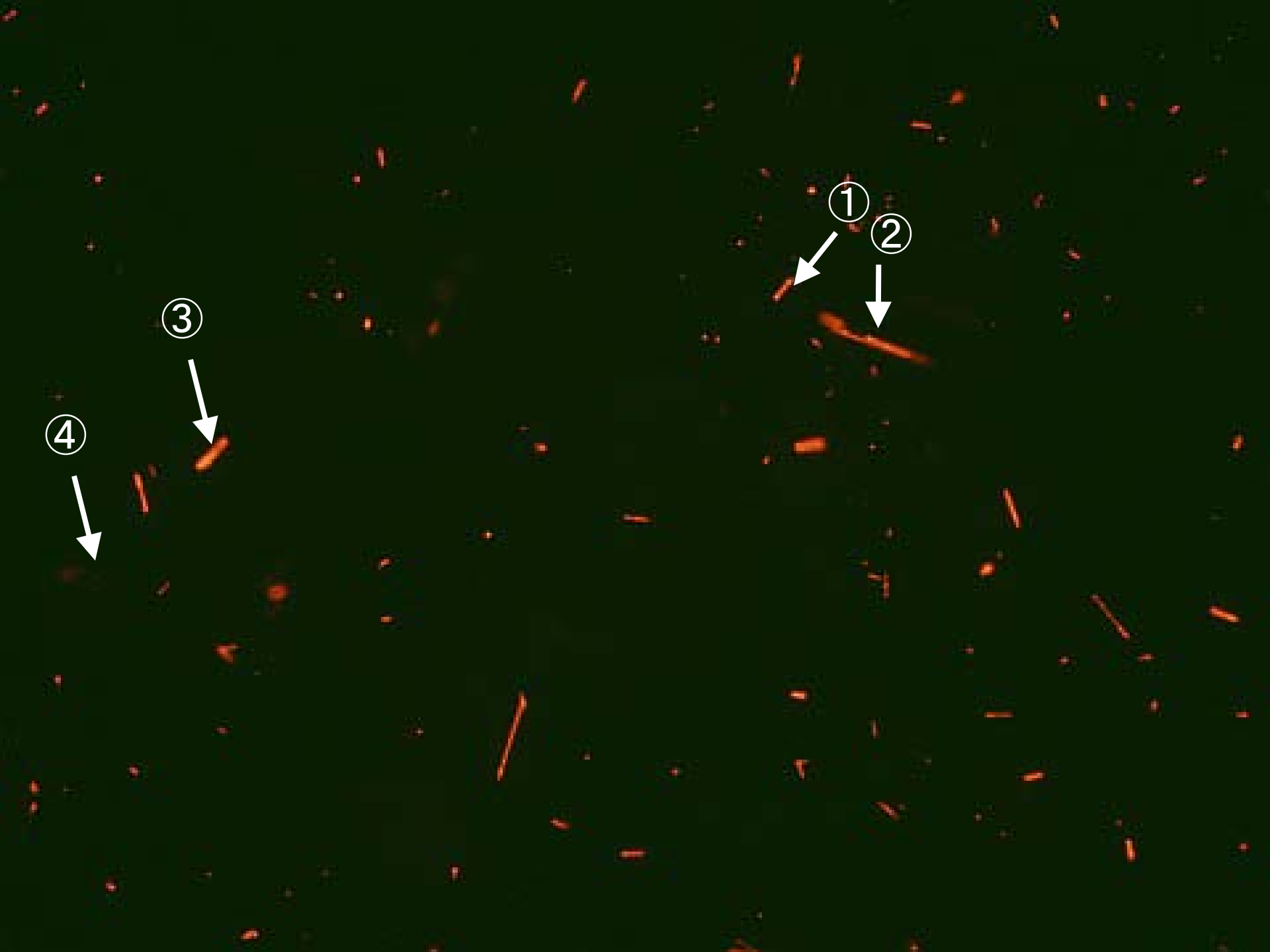
Fluorescent microscopy



Light for FM

Light for PCM


①
②
③
④


①
②
③
④


①non-asbestos
②asbestos
③asbestos
④non-asbestos

Detection of Airborne Asbestos by Fluorescent-labeled protein probe and its Application to quick Monitoring

1. We discovered asbestos-binding proteins.
2. We developed a fluorescence microscopy-based method for selective and highly sensitive detection of two different types of asbestos.
3. The diameter of the thinnest asbestos fibers visualized under fluorescence microscopy was 30-35 nm.
4. Then we proposed PCM and FM fusion analysis.
5. This method could be used for on-site quick monitoring of airborne asbestos, for example, during demolition work.